THE FORMATION OF GERM LAYERS IN ACTINIA BERMUDENSIS VERR.

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While at the Bermuda Biological Station during the summer of 1909 I attempted to secure material for a study of the development of *Actinia bermudensis* Verrill, a viviparous actinian that is abundant between tide marks in the shaded limestone caverns along the shores of the smaller islands. Since this material proved to be lacking in the earliest stages in the development, so that the complete embryology could not be worked out, I have thought it advisable to publish the following account as it covers one of the most unsettled points in the development of anthozoans.

According to McMurrich (1891), the only well authenticated cases among the Cœlenterata in which the endoderm arises by invagination are those of *Nauthisoe* and *Pelagia*, both Scyphomedusæ. Jourdan's (1878) account of the formation of the endoderm in *Actinia aquina* he discredits on the basis of his own observations on *Metridium* (*marginatum*) *dianthus*. Kowalevskis's (1873) account of the process in *Adamsia rondeletti*, which in the original was inaccessible alike to Professor McMurrich and myself, he dismisses in the same manner saying that it was probably an error in interpretation.

Appellöf (1900) describes the endoderm formation in two species of actinians: *Urticina* (*Tealia*) *crassicornis* and *Actinia aquina*. His observations on the latter species confirm the opinion of McMurrich, namely, that there was no true invagination. In the case of *Urticina*, on the other hand, he describes and figures a true invagination, which from his figures could not be considered as an error of interpretation, since he worked with serial sections.

Faurot (1907) has described a true although rather unusual type of invagination in the development of *Sagartia parasitica* and *Adamsia palliata*.

In *Metridium*, according to McMurrich, the result of segmentation is the formation of a hollow blastula with a considerable cavity. Later the inner ends of the cells are constricted off—by the appearance of vacuoles in the line where the separation is to occur—to form the endoderm. At the time when this process is finished there appears at one pole of the blastula a slight de-

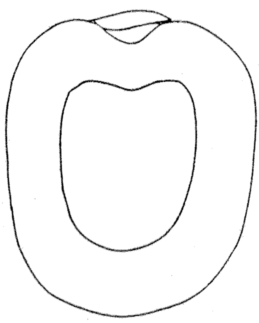

FIG. 1. Pseudogastrulation in *Metridium*. After McMurrich.

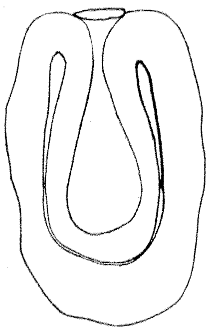

FIG. 2. Later stage in the pseudogastrulation of *Metridium*. After McMurrich.

pression which gives the embryo, when it is seen in optical section, an appearance similar to the early stages of a true invagination (Fig. 1).

When the mouth has broken through, the resemblance to an invaginate gastrula is even more complete, so that until such embryos had been seen in sections it would be almost certain to mislead any observer. In reality, however, the two layered condition had been reached before the mouth was formed. This so called "Pseudo-gastrula" McMurrich held to be the true condition in those forms in which invagination had been reported to occur.

All the material of *A. bermudensis* was obtained by slitting the adult individuals longitudinally, and then washing the embryos into a dish of sea water with the stream from a pipette. All stages including the young in which the second series of tentacles was complete, and which were ready to be liberated from the body of the parent were capable of swimming about actively by means of their cilia.

The earliest segmentation stages were never found among the

material obtained by washing out the adults, nor did sections of the mesenteries of the adult show any segmenting eggs, so the processes leading up to the formation of the blastula cannot be considered. In the section represented in Fig. 3, the blastula

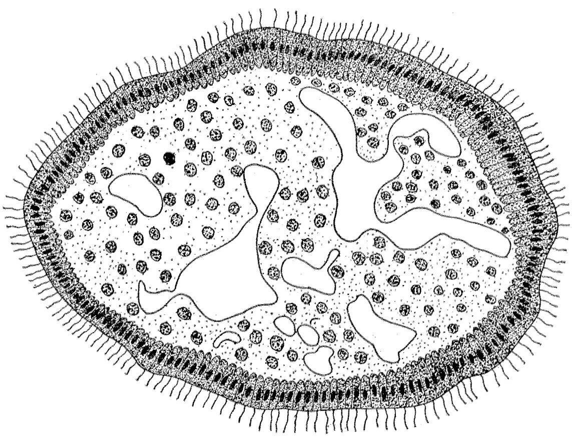

FIG. 3. Early blastula of *A. bermudensis*. Leitz 1/12 and no. 2 (reduced).

is completely formed. The cells are very numerous and of practically the same height over the entire circumference of the blastula. The interior of the blastula is to a great extent filled with a comparatively thin, lightly staining, plasma-like material, in which there are many yolk granules.

The manner in which the separation of the yolk spheres took place during the segmentation can be conjectured only, but, from the appearance of the stage just described it would seem probable that, just as in *Urticina*, there is never an extensive blastula cavity. Instead the yolk material is probably separated from the cytoplasmic portion of the cells in an early stage of segmentation.

In a later stage (Fig. 4) the blastula has become more elongated. The cells have become relatively thinner, and higher, while at one pole there is the first indication of the depression that marks the beginning of the infolding of one portion of the blastula wall to form the endoderm.

Within the interior of the blastula, there has been a marked increase in the relative amount of space unoccupied by nutritive

materials. The plasma-like substance has disappeared for a considerable proportion and the yolk spheres have undergone an apparent disintegration. Their outlines are no longer distinct, as in the earlier stages, and in many instances, they can be ob-

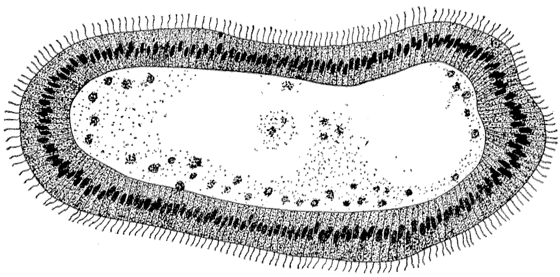

FIG. 4. Blastula of *A. bermudensis* just at the beginning of the invagination.

served breaking up into rather coarse granules which are being spread out through the surrounding plasma-like material.

In the stage shown in Fig. 5 the process of invagination has gone on until the section shows a well-marked early gastrula, and puts beyond any question the type of endoderm formation in this species. The character of the cells in the invaginating

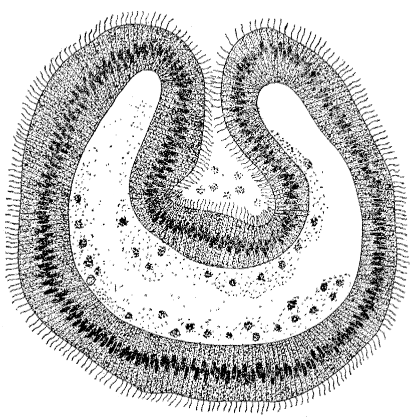

FIG. 5. Invagination of *A. bermudensis.*

area has not as yet undergone any change to distinguish them structurally from those making up the ectoderm.

The nutritive material within the original blastocœl shows practically the same conditions as noted for the stage last de-

scribed. Within the gastrula cavity there have appeared some few masses that are apparently composed of the rather coarse granules that come from the breaking up of the yolk spheres.

In the older gastrula, Fig. 6, almost all of the nutritive material has come to lie within the gastrocœl, only a comparatively few of the masses of granules, resulting from the disintegration of the yolk spheres, remaining in the original blastula cavity.

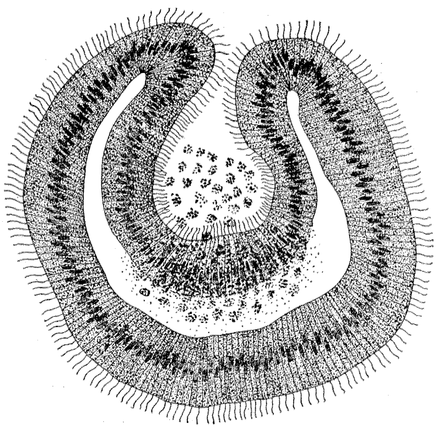

FIG. 6. Later invagination stage. *A. bermudensis.*

Appellöf (1900) describes the same sort of migration of the nutritive material during the invagination of *Urticina.* He raises the question as to whether the yolk material seen in the gastrocœl after the invagination is complete is the same as that seen in the blastocœl before invagination, and if so how the transfer could take place. Of the two possibilities in the way of a transfer he mentions: first that the yolk spheres might be absorbed by the cells of the invaginating layer and then be set free to collect in the gastrocœl; and secondly that the yolk elements pass ("sich drangen oder gedrängt werden") between the cells of the invaginating layer. According to his interpretation, his material supports the last mentioned view as many of the cells appear shrunken in diameter while yolk spheres may be seen between such cells separating their lateral walls one from another. He mentions besides that the walls at the inner ends of the cells are often very indistinct during the time when this process is

going on, although over the remainder of the gastrula wall there is no appreciable change in the characteristics of the cells.

Appellöf makes no direct mention, although his Fig. 13, Pl. 2, shows, that while the transfer of the nutritive material is taking place the yolk spheres are breaking up rapidly, so that to infer that the spheres pass through the invagination layer in their original condition is unnecessary. In the older gastrulæ of *A. bermudensis* a complete yolk sphere was scarcely ever found in the gastrocœl. While many of the masses of nutritive material still retained their identity and practically their original volume it could be observed in every instance, that the sharp outline was no longer apparent, and usually the granules were separated from one another. It is also noticeable in the section shown in Fig. 6, that nearly all of the yolk material present in the blastocœl is massed about the invaginating cell layer and that the inner ends of these cells are much less sharply defined than they were in the younger stages, Fig. 5, or than they are more laterally in the invaginating area. The central part of the invaginating area is more densely filled with granules than are those parts farther to the sides. The granules in this denser area are also markedly larger than the granules in the cytoplasm of the more laterally placed cells.

It seems, then, beyond question that in *A. bermudensis*, just as in *Urticina crassicornis*, there is an actual passing of the yolk material, in a practically unaltered state, through the layer of invaginating cells to the forming gastrocœl. As the cells of the invaginating layer approach those of the outer gastrula wall all of the yolk passes through so that the two layers come into contact and the supporting layer is secreted between them.

In an older embryo, Fig. 7, in which the formation of the stomodeum has begun, the gastrocœl—gastro-vascular cavity—still contains a considerable amount of the yolk material which now appears as distinct granules. In nearly all instances, however, the granules are arranged in groups which show clearly their origin from an originally more circumscribed mass.

In this last-mentioned stage the cells making up the endoderm have undergone a considerable change so that their histological characteristics are very different from those of the ectoderm cells.

which have retained very nearly the appearance of the original blastula cells. The endoderm cells have now become much broader in proportion to their height, their cytoplasm is much less dense than formerly, and takes the stains less readily, while the nuclei have become markedly less conspicuous. In some regions, where the body of the embryo is most contracted, especially about the region of the forming stomodeum, the outlines of the endoderm cells are very indistinct, and indeed impossible to make out at the proximal ends of the cells.

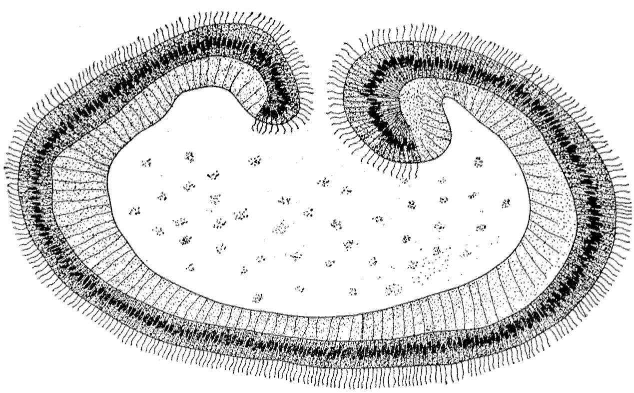

FIG. 7. Young embryo in which the stomodeum is forming. Leitz 7 and 2.

In regard to the interpretation of the optical sections shown in McMurrich's Fig. 10, Pl. XIII., it is interesting to compare my Fig. 6, with the figures of the embryos of *Actinia aquina* given by Appellöf. In the last mentioned form, where the endoderm arises by delamination, there occurs at the time of the breaking through of the mouth opening a decided thinning of both germ layers at the point where the mouth opening will appear. When the mouth has been formed the tissues are very thin all around it, and in a longitudinal section there appears about the mouth this thin area instead of the more thickened area which is found about the gastropore of an invaginate gastrula. So that in some instances at least, McMurrich's criticism of the interpretation of optical sections of whole mounts would not apply.

REFERENCES.

Appellöf, A.

'00 Studien uber Actinien-Entwicklung. Bergens Museum Aarbog for 1900.

Faurot, L.

'07 Nouvelles Recherches sur le Developpement du Pharynx et des Cloissons chez les Hexactinies. Arch. d. Zool. Exper. et Gen., Ser. 4, T. 6, 1907.

Jourdan, E.

'78 Recherches Zool. et Histolog. sur les Zoanthiares du Golfe de Marseille. Ann. Sci. Nat., Ser. 6, T. 10, 1878.

McMurrich, J. P.

'91 Contributions to the Morphology of the Actinozoa, II. On the Development of the Hexactinæ. Jour. of Morphology, Vol. 4, 1891.